Can you clone a gene?

*\*\* This activity was adapted from a posting by Kim Foglia*.

In this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc18 plasmid) can then be used to transform bacteria so that it now expresses a new gene and produces a new protein. These diagrams are on the last page of this activity to be easily removed and manipulated.

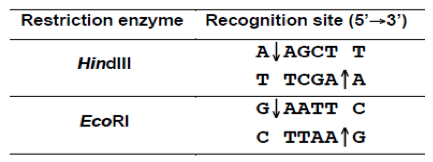
1. From the white paper, cut out the puc18 plasmid DNA in a long strip.

2. Attach the ends together with tape to make a loop to simulate the circular DNA of a plasmid.



3. From the green paper strip, cut out the Jellyfish Glo gene DNA. Leave it as a straight strip. (This is a gene from a vertebrate not a bacterium, so it is not circular.) Think about how you will get this DNA into the circular plasmid.

The start and stop sequences for transcribing the Jellyfish GFP or Glo gene are highlighted. These are needed to transcribe the gene properly when it is read. In addition, the HindIII & EcoR1 restriction enzyme cutting sites (sequences of bases) are marked in bold on the Jellyfish Glo gene DNA. The two restriction enzymes and their respective restriction sites are listed below. These enzymes act as “molecular scissors” to cut the DNA at these sequences in the DNA:



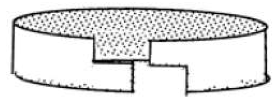
The 6-letter sequence represents the nitrogen base sequence that the enzyme recognizes, and ↑ represents the place where the DNA will be cut by the enzyme. For example, *Hind*III cuts between A and A whenever it encounters the 6-base sequence AAGCTT.

4. Cut the green Jellyfish DNA as if you have used the restriction enzyme, *Hind*III. Be sure to leave “sticky ends.”

5. Also, cut the white puc18 plasmid DNA as if you have performed a restriction enzyme digest with *Hind*III. Be sure to leave “sticky ends.”

6. Now incorporate the green Jellyfish Glo gene into the white plasmid. Attach the sticky ends of the Jellyfish Glo gene to the sticky ends of the puc18 plasmid and seal with “molecular glue”, the enzyme ligase (tape will be used in our lab).

7. You have successfully cloned a gene! You now have a single plasmid with a new gene and can use that to transform a single bacterium. The bacterium will now make green Jellyfish glow protein and will glow under black light. Wear your transgenic bacterial plasmid proudly on your head. ☺



*Questions you should be able to verbally answer are on the back……*

1. What is a plasmid?
2. What are restriction enzymes used for in nature?
3. What is meant by “sticky end?”
4. Why did we cut both segments of DNA with the same restriction enzyme?
5. Why did we make sure to include the start and stop DNA sequences for the Jellyfish GLO gene in our cut segment?
6. What would have happened if we had cut both the Jellyfish Glo gene and puc18 plasmid with the EcoR1 restriction enzyme?
7. If we want to now produce a lot of this Jellyfish Glo protein, what do we have to do after this first successful cloning to reach our goal?
8. What are some current uses of this technology in medicine?